The NMDA receptor complex: A long and winding road to therapeutics

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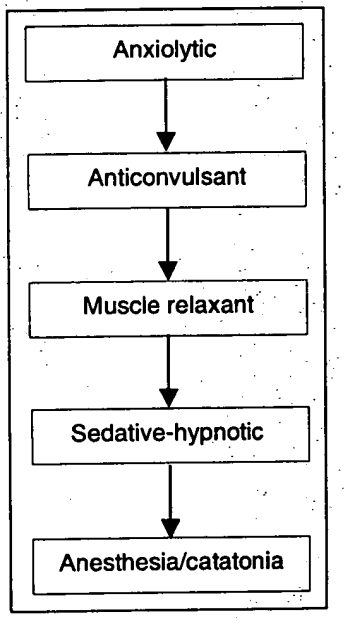
Advances in our basic understanding of inhibitory and excitatory amino acid neurotransmission have provided the foundation for directed drug discovery programs to modulate inhibitory GABAergic and excitatory N-methyl-D-aspartate (NMDA) receptor-mediated synapses. γ-Amino butyric acid ($GABA_A$) and NMDA receptors are complex ion channels formed by multiple protein subunits that act as binding sites for transmitter amino acids and as allosteric regulatory binding sites to regulate ion channel activity. In the case of the NMDA receptor complex, one such allosteric site binds the obligatory glycine and/or D-serine co-agonist. Historical data from preclinical and clinical studies of GABAergic agents have clearly demonstrated that direct receptor modulators lack sufficient therapeutic indices to warrant clinical utility. However, pharmacological modulation of allosteric sites of the GABA multimeric receptor has resulted in the clinical development of safe and efficacious agents, exemplified by the benzodiazepines. Research has also revealed a similar outcome for the NMDA receptor, with allosteric modulators demonstrating improved safety profiles in the modulation of excitatory amino acid (EAA) transmission compared with direct NMDA receptor antagonists. First-generation EAA drugs were low affinity channel blockers of the NMDA multimeric receptor complex and included the anesthetic agent ketamine and the Alzheimer's drug memantine. As predicted by preclinical studies, direct NMDA receptor antagonists (eg, selfotel (Novartis AG)) and high-affinity channel blockers (eg, dizocilpine) failed in the clinic as a result of narrow therapeutic indices. More recent efforts have focused on glycine/D-serine co-agonist function. These approaches include partial glycine agonists, in their agonist dose-range, for cognitive improvement and for treating schizophrenia. Such partial glycine agonists are also being advanced for the treatment of neuropathic pain in the antagonist dose range. An alternate approach to partial glycine agonists is to inhibit the uptake carrier(s) for glycine (ie, GlyT-1 and GlyT-2), thereby potentiating the lifetime of synaptic glycine. A number of glycine uptake inhibitors have been reported and their preclinical profiles support investigation into their utility in treating schizophrenia.

Keywords Glycine partial agonists, GlyT-1 uptake inhibitors, neuropathic pain, NMDA receptor, schizophrenia, stroke

Scientific background

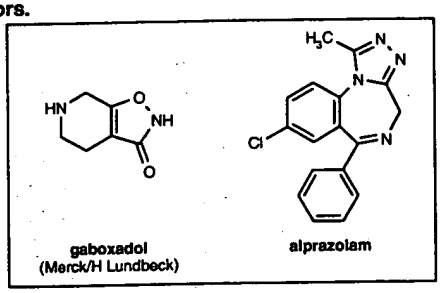
While there are a number of excitatory amino acid (EAA) receptor subtypes present in the central nervous system (CNS), the *N*-methyl-D-aspartate (NMDA) receptor subtype has received the greatest investment of resources with regard to drug discovery, largely because of its demonstrated roles in a vast array of CNS functions [1]. However, this wide range of CNS functions becomes a complicating issue when developing an NMDA receptor modulator that is both clinically efficient and lacks serious CNS side effects. Accomplishing this has been particularly challenging since significant disruptions of the dynamic balance between EAAs and the inhibitory amino acid neurotransmitter γ-amino butyric acid (GABA) result in a spectrum of CNS side effects that range from mild to serious. The overall pharmacological profile of positive modulators of the $GABA_A$ receptor and negative modulators of the NMDA receptor is a continuum, based on increasing drug concentrations (Figure 1).

Figure 1. Continuum of pharmacological actions of increasing doses of $GABA_A$ receptor agonists and NMDA receptor antagonists.



The complexity of developing safe pharmacological modulators of amino acid receptors has historically been well documented for GABA. For example, the search for positive modulators of this receptor led to the development of direct $GABA_A$ receptor agonists, such as gaboxadol (THIP, Merck & Co Inc/H Lundbeck A/S; Figure 2), however, the majority of these compounds failed upon reaching the clinic as a result of narrow therapeutic margins [2]. Alternatively, barbiturates, which are modulators of an allosteric site on the $GABA_A$ complex, demonstrated significantly improved therapeutic margins, although their use was limited due to issues concerning respiratory depression and abuse. Another class of allosteric modulators of the $GABA_A$ multimeric complex, the benzodiazepines (eg, alprazolam (Figure 2)), not only displayed superior therapeutic margins compared with direct agonists but also proved to be safe, except for a potential for abuse that was associated with chronic use (Table 1).

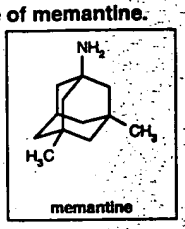
Figure 2. The structures of selected $GABA_A$ receptor modulators.



In the case of EAA systems, researchers have directed significant resources to discovering and developing NMDA receptor antagonists. In preclinical studies of NMDA receptor antagonists, a number of potential clinical limitations were revealed, for example: ataxia and catatonia; sedation; psychotomimetic actions and confusion; hypertension; and cerebral cortical vacuolization involving cytoplasmic vacuoles in limbic cortical, potentially leading to neuronal necrosis [3-5,6•,7].

As predicted by preclinical studies, and in analogy to $GABA_A$ receptor modulators, direct competitive NMDA receptor antagonists (Table 1) exhibited insufficient therapeutic margins for human use when evaluated in extensive clinical trials [8-10]. Similarly to the barbiturate modulation of the $GABA_A$ receptor, non-competitive NMDA receptor blockers demonstrated improved therapeutic margins and resulted in the introduction of several marketed products. These included ketamine, for anesthesia, and memantine (Figure 3), for the treatment of memory loss associated with Alzheimer's disease (AD) [11•], both of which are low-affinity, non-competitive inhibitors of ion channel function in the NMDA receptor complex. In contrast, high-affinity non-competitive NMDA receptor antagonists, like competitive receptor blockers, were limited by pyschotomimetic side effects (eg, agitation, abnormal dreaming, paranoia and hallucinations) [9,10]. The side effect of hypertension [9,10] appeared to be confined to high-affinity non-competitive NMDA receptor antagonists, and cortical vacuolization [7] was reported for both competitive and high-affinity non-competitive NMDA receptor antagonists.

Figure 3. The structure of memantine.



Potential clinical advantages for glycine site modulators

The glycine binding site of the NMDA receptor complex is classified as a co-agonist site with affinity for the endogenous ligands glycine and D-serine [12,13]. As an allosteric site it has been extensively investigated as a potential target for generating drug candidates with improved safety profiles [1,14]. This led to the discovery and characterization of a number of competitive glycine antagonists and partial glycine agonists [14-16]. The advantage of partial glycine receptor agonists resides in their ability to block excessive NMDA function while also potentiating NMDA receptor function in the case of abnormally depressed NMDA-mediated neurotransmission. The partial glycine receptor agonists therefore represent the ideal profile for an NMDA receptor drug candidate and should not produce a large imbalance between GABAergic and EAA systems.

The improved safety profile of glycine receptor antagonists and partial glycine receptor agonists compared with competitive and high-affinity non-competitive NMDA receptor antagonists is reflected by: (i) lack of phencyclidine-like behavioral effects [17] and lack of stimulation of limbic psychotomimetic actions in humans [9,10]; (ii) lack of dopamine turnover [18], which translates into lack of

Table 1. Comparative table of $GABA_A$ and NMDA receptor modulators.

Therapeutic index	GABA receptor modulators	NMDA receptor modulators
Low	Direct agonists • Muscimol • Gaboxadol	Competitive antagonists • Selfotel • CPP
Intermediate	Allosteric modulators: Barbiturates • Phenobarbital • Secobarbital	Allosteric modulators: Non-competitive antagonists • Aptiganel (Oregon Health Sciences University/ CeNeS Pharmaceuticals Inc) • Dizocilpine
High	Allosteric modulators: Benzodiazepines • Alprazolam • Diazepam	Allosteric modulators: Glycine antagonists • Gavestinel • Licostinel Allosteric modulators: Glycine partial agonists • D-Cycloserine • L-687414 • NT-13

CPP 3-((±)-2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid.

Table 2. Selected examples of drugs that have demonstrated preclinical efficacy but have failed in the clinic.

Compound	Action	Reference for preclinical efficacy	Reference for clinical failure
Clomethiazole	$GABA_A$ modulator	[21]	[9,10]
Selfotel	Competitive NMDA receptor antagonist	[6•]	[9,10]
Aptiganel	Non-competitive NMDA receptor antagonist	[6•]	[9,10]
Gavestinel	Glycine receptor antagonist	[22]	[9,10]

psychotomimetic activity in humans [9,10]; and (iii) lack of cortical vacuolization [19,20]. With regard to these issues, the therapeutic areas of stroke, neuropathic pain and schizophrenia will be reviewed. NMDA receptor antagonists have failed as therapeutic agents for the indication of stroke, however, NMDA receptor modulators may still offer medical breakthroughs in the clinical areas of neuropathic pain and schizophrenia.

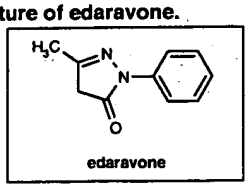
Clinical failure: Stroke

Both GABAergic compounds and NMDA receptor antagonists demonstrated neuroprotective potential against ischemic stroke in preclinical models, but failed in the clinic. Table 2 provides selected examples of such drug candidates.

While these failures indicated that NMDA receptor modulators will probably never be effective treatments for stroke, a substantial amount of data was generated defining the optimal preclinical criteria for a stroke therapeutic. These criteria include: efficacy in both the transient and permanent rat middle cerebral artery occlusion (MCAO) models of brain ischemia; a therapeutic window of ≥ 6 h in these models; verification of permanent functional recovery and not a transient delay in neuronal deficits; and significant safety margins to allow optimal dosing for intravenous infusions.

The only drugs to meet these criteria were not NMDA receptor modulators but were instead the antioxidant edaravone (Figure 4), which was launched in Japan in June 2001 [23], and the spin-trap (regenerating free radical scavenger) cerovive (AstraZeneca plc/Renovis Inc), which is currently in international phase III clinical trials. Futhermore, cerovive protected mitochondrial integrity in rat models of focal cerebral ischemia, hence maintaining energy reserves and blocking activation of the caspase cascade associated with efflux of cytochrome C from compromised mitochondria [24].

Figure 4. The structure of edaravone.



Potential clinical utilities

Neuropathic pain

NMDA receptor antagonists inhibited noxious sensory transmission in the dorsal horn of the spinal cord. These actions were demonstrated in a number of preclinical analgesia assays, initially with competitive NMDA receptor antagonists and then subsequently with non-competitive NMDA receptor antagonists [25,26]. However, this inhibition was consistently observed at doses that were also ataxic. The lack of therapeutic margin does not appear to be an issue with the low-affinity non-competitive NMDA receptor antagonist memantine, which demonstrated efficacy in both preclinical pain models [25,26] and in clinical trials with patients experiencing neuropathic pain [27].

Glycine receptor antagonists, such as L-701324 (Figure 5) and MDL-29951 (sanofi-aventis; Figure 5), and partial glycine receptor agonists, such as D-cycloserine (DCS), L-687414 and HA-966 (all Figure 6) were active in neuropathic pain models and also demonstrated superior therapeutic margins in relation to competitive and high-affinity non-competitive NMDA receptor antagonists (Table 3).

Figure 5. The structures of selected glycine antagonists.

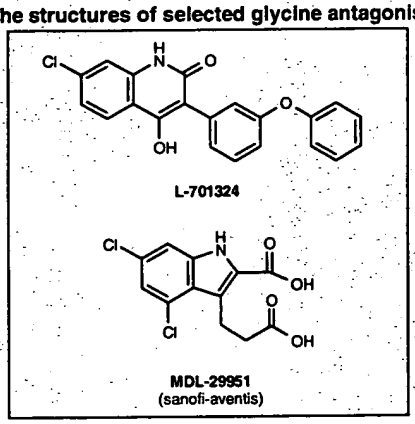
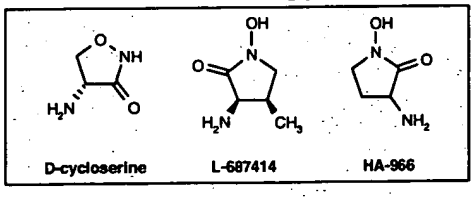


Figure 6. The structures of selected glycine partial agonists.



DCS is a broad-spectrum antibiotic, usually administered at a dose of 250 to 500 mg twice-daily for the treatment of tuberculosis. At much lower doses, DCS is also a potent NMDA receptor modulator. Preclinical data with DCS in rat models revealed a narrow glycine agonist dose range (1 to 10 mg/kg sc) and a wide glycine antagonist dose range above these concentrations [32]. In clinical trials, DCS

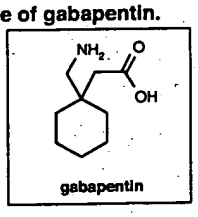
Table 3. Selected examples of glycine receptor antagonists and partial glycine receptor agonists.

Glycine receptor antagonists	Glycine receptor partial agonists
• L-701324 [28,29] • MDL-29951 [29] • 5,7-Dichloro-2-dihydroxy-3-phenyl-quinoline dione [29]	• D-Cycloserine [29] • HA-966 [29,30] • L-687414 [28,29] • NT-13 [31]

was administered twice-daily to AD patients at doses of 5, 15 or 50 mg, with results demonstrating that the 15-mg dose (0.2 mg/kg) improved implicit memory in patients [33]. These data were not substantiated in follow-up clinical trials [34]. However, these were difficult clinical trials to conduct since the agonist dose range of a partial glycine agonist (eg, DCS) is narrow and may not be wide enough for dosing a heterogeneous patient population, as in AD studies. The use of partial agonists in their antagonist dose range is much simpler with regard to both clinical design and dose-ranging. Currently, NT-13 (Nyxis Neurotherapies Inc) [31], a peptide (Thr-Pro-Pro-Thr), appears to be the only partial glycine agonist entering the clinic to demonstrate potential for a neuropathic pain indication.

The leading product for the treatment of neuropathic pain is the anticonvulasant gabapentin (Figure 7), which was launched by Pfizer Inc for the treatment of epilepsy and is currently registered for the indication of neuropathic pain [35]. The exact mechanism of analgesic action of gabapentin is not yet known, however, in neuropathic pain models (formalin late-phase response [36-38], substance P-induced thermal hyperalgesia [39,40] and tactile hyperalgesia with thermal injury [41]), the analgesic effects of the drug were dose-dependently antagonized by D-serine, an agonist of the NMDA-associated glycine receptor. This suggested that the analgesic actions of gabapentin in these models were dependent upon decreases in glycine-mediated NMDA receptor neurotransmission. Since gabapentin has no affinity for the various sites on the NMDA receptor, the compound most likely acts upstream of the NMDA receptor in a series of interlinked actions that ultimately require decreased NMDA-mediated neurotransmission for analgesic efficacy.

Figure 7. The structure of gabapentin.



gabapentin

Although gabapentin has demonstrated clinical efficacy in the treatment of neuropathic pain, the reduction in pain score is generally 2.05 points on an 11-point numerical rating scale [42]. It is expected that a direct modulator of the NMDA-associated glycine site might offer greater efficacy and possibly a superior therapeutic index to gabapentin. NT-13, which is entering phase I clinical trials, will be the first to test this hypothesis.

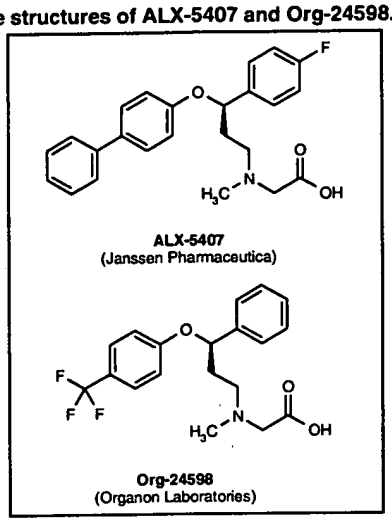
Schizophrenia

A decrement in EAA tone within limbic cortical regions has been hypothesized to play a role in the etiology of schizophrenia, based on data from preclinical studies of actions of antipsychotic agents in models of EAA hypofunction and data from studies of the clinical psychosis induced by non-competitive NMDA receptor blockers in humans [43-48]. Early research to potentiate NMDA-mediated neurotransmission has focused on the application of partial glycine agonists because these compounds would lack 'excitotoxic' potential. Indeed, since partial glycine agonists cannot induce full efficacy at the co-agonist site they offer a significant intrinsic safety feature. Furthermore, if excessive NMDA-mediated transmission were present at a given synapse, the partial glycine agonist would act as an antagonist and decrease NMDA receptor tone to reach the physiological range. Following the identification of the co-agonist site on the NMDA receptor, a plethora of publications negated the importance of this binding site, particularly as the micromolar concentrations of glycine observed in the cerebrospinal fluid were proposed to be capable of saturating glycine receptors *in vivo*. Unfortunately, these publications did not consider the rich literature history that establishes the principles of metabolic compartmentation within the synaptic cleft, which is key to limiting neurotransmission and maintaining point-to-point communication in the CNS. A review of the *in vivo* behavioral and neurochemical data that rebutted these misconceptions was first published in 1995 [49•] and the fact that the co-agonist site is not saturated *in vivo* has since been further validated using electrophysiological approaches [50,51]. The methods that have been investigated with the intention of potentiating NMDA neurotransmission in schizophrenia patients have included oral treatment with the partial glycine receptor agonist DCS [52,53], oral loading with glycine [53,54] or D-serine [55] and oral treatment with the glycine uptake inhibitor sarcosine [56].

These techniques appeared to provide clinical benefit with regard to negative symptoms and cognitive deficits that are observed in schizophrenia patients, except when used in combination with clozapine therapy [57]. The full benefit of this new approach requires more specific and/or more potent drugs for clinical testing. In this respect, extensive research into glycine uptake inhibitors is ongoing, with both glycine transporter type 1 (GlyT-1; ie, astrocytes) and GlyT-2 (ie, axons and presynaptic terminals) cloned and characterized [58]. Additionally, potent inhibitors of GlyT-1 (eg, ALX-5407 (Janssen Pharmaceutica NV; Figure 8)) [59-65] and GlyT-2 (eg, Org-24598 (Organon Laboratories Ltd; Figure 8)) [66,67] have been reported. The drawbacks to these first-generation glycine uptake inhibitors are their poor pharmaceutical properties and their potential to

increase glycine concentrations in both Glya and Glyb synapses, but the net consequences of this lack of specificity remain to be examined in more detail.

Figure 8. The structures of ALX-5407 and Org-24598.



Conclusion

In the last 20 years extensive resources have been devoted to characterizing the various components of the NMDA macromolecular receptor complex. The overall result of these efforts has led to the introduction of several low-affinity non-competitive NMDA receptor antagonists into clinical use. Future drug candidates appear to reside with the development of partial glycine agonists and glycine uptake inhibitors for the potential treatment of neuropathic pain and schizophrenia. For a recent review of the potential utility of NMDA receptor subtype modulators see reference [68].

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